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Evaluation of Effective Concentration of Honey Through Bio-Assay of Eri Silkworm, *Samia Cynthia Ricini*boisduval

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Abstract: Ericulture is mainly confined to North-eastern states of India since the time ancient time of an integral part of the local tribals. Ericulture, which is traditionally rearing of the eri silkworms (*Samia Cynthia ricini*) primarily for pupae as food purposes and silk fabric for their family use. The main hindrance faced was production would be an uneconomical because of the similar food habits. Here, eri silkworms were reared using their primary food Castor leaves (*Ricinus communis*) along with the supplementation of honey and the performance of economic parameters and the changes in bio-chemical compositions*viz.*, Protein and Carbohydrate content in different tissues *viz.*, Haemolymph, Fat body & Silk gland were recorded in 4th and 5th instar larvae. Leaves with different concentration 1%, 2%, 3%, 4%, 5% of honey was dipped and dried under the shade, along with distilled water dipped and normal leaves as controls were fed for 4th and 5th instar Eri silkworms and the economic and nutritional results were recorded. Larvae fed with 5% concentration showed the highest nutritional contents like protein and carbohydrate, followed by 4% to 1% over controls in Haemolymph, fat body and silk gland. As well quantitative traits like larval weight, cocoon weight and pupal weightwere also exhibited increase in their quantitative character in 5% subsequently 4% to 1% above controls but in case of shell weight, shell ratio and silk productivity 3% showed highest weight. The research findings presented in the study suggest or hint at a particular conclusion, that castor leaves with the honey is the suitable diet for *Samia Cynthia ricini*to escalate the quantitative and qualitative characters.

Keywords: *Castor Leaves, Bio-Chemical, Eri Silkworm, Quantitative Trait, Samia Cynthia Ricini.*

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I. INTRODUCTION

Silk is the most luxurious textile fiber in the world with unparalleled grandeur, natural sheen and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and known as the "*Queen of Textiles*" the world over. On the other hand, it stands for livelihood opportunity for millions of people with low capital investment and highly remunerative cash crop. As it is rural based and generate employment with on and off-farm activities captivated the planners and policy maker's attention to develop socio-economically like India (Anonymous, 2015).

There are two different sectors in Sericulture industry, mulberry and non-mulberry (Vanya). Mulberry which is mainly concentrated on silk production from silkworm *Bombyx mori* L., whereas, non-mulberry sericulture divided into three kinds of silks Tasar, Eri and Muga, which has the varieties of species from Lepidopteran order and Saturnidae family *viz.*, *Antheraea mylitta* Drury (Tasar), *Samia Cynthia ricini* Boisduval and *Antheraea assamensis* respectively.

Similar to mulberry silkworms, Eri silkworms are also popular and domesticated, while mulberry silkworm is monophagous and has three voltinism characters, Eri is polyphagous and multivoltine in character. The word "Eri" is derived from the Sanskrit term "Erranda", which refers to the castor plant *Ricinus communis* L., which is the primary host plant. However, Eri silkworms also feed on other 30 plant species as a secondary food plant (Arora and Gupta, 1979).

With respect to Eri silk production in the world, India is the largest producer, ranks first and contributes 96% of total Eri silk production. As Eri silkworm feeds on many food plants as primary and secondary, all food plants show variations in growth and development. Mainly Eri food plants can observe abundantly in natural forests of plains

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and hilly areas/region. These plants availability may vary with seasons for Eri silk production. Food plants can be interchanged based on the availability during scarcity of the one host (Patil and Savanuramath, 1994).

Production of a good crop directly depending on the quality of leaves provided. Growth, development and cocoon yield are mainly influenced by the castor genotype and the quality of the leaves fed to the silkworms. Survival capacity of non-mulberry silkworms mainly influenced by the nutritional status of the leaves (Pandey, 1995).

Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the life and behavior of different insects (Rajasekaragouda *et al.*, 1997). As silk is the main product in sericulture, test has been reported that, supplementing the various extracts of the medicinal plants will influence the silk filament length, silk gland weight and body weight of *Bombyx mori* L. (Murugan *et al*., 1998). "The principles of co-operating supplements" reported to judge the research importance on effect of different fortification agents in silkworm nutrition (House, 1966).

Foliar application of plant growth substances is one of the quicker techniques for improvement of leaf productivity and effectively uptake nutrients. Several studies also reported the effect of foliar spray with respect to vegetables and cash crops (Setua *et al*., 2009). Healthy growth and development of silkworm *Bombyx mori* L. directly proportional to the nutrition values of the leaves. Producing a good quality cocoon and getting a quality silk is majorly influenced by the larval nutrition and nutritive value of mulberry leaves (Legay, 1958; Seki and Oshikane, 1959).

Silkworms need specific essential sugars, amino acids, proteins and vitamins for the survival and growth as well to improve the silk gland growth. (Sengupta *et al*., 1992). Significant seasonal variations occur in the nutritional value, composition of mulberry leaves and silkworm cocoon production depends on various factors *viz*., weather, pests and diseases (Ito, 1964).

In silkworms, silk fibroin is derived mainly from 4 amino acids: alanine, serine, glycine and tyrosine, which come from their dietary source of protein and amino acids (Kirimura, 1962&Ito, 1983). As amino acids play an important role in glucose, tryptophan and organic acid metabolism in silkworms, 72-86 % of amino acids from mulberry leaves will obtained and 60 % of the absorbed amino acids will be utilized in the production of silk. (Lu and Jiang, 1988).

With the added additives to the mulberry leaves, some quantitative traits *viz.*, cocoon percentage, low larval mortality, fecundity and hatchability will improve without affecting on larval duration or fitness. Vitamin C & B as coenzymes in amino acid metabolism and anti-oxidant agents acts on the larval tissue concentration and show the improved development and productivity of larvae (Babu *et al*., 1992).

Vitamin-B complex found to be significantly beneficial with respect to larval grown and development as well cocoon characters. This character is mainly due to the efficient conversion of dietary nitrogen to form a cocoon shell. On the other hand, silk economic character may also increase due to protein conversion efficiency of silk gland with the result of increased availability of vitamins and the positive anti-bacterial effect of honey on the growth of many kinds of bacteria (Suprakash and Pal 2002).

Studies have been found that, supplementing the amino acid results improvement in the silk quantity. Thus, comprehensive effort was made to determine whether supplementation of secondary metabolites like lower concentrations of pectin, proline and amino acid mixture influences the growth and development of silkworms as well cocoon character (Etebari and Matindoost, 2005).

In sericulture industry quality of mulberry leaves plays an important role, hence many researches were conducted to develop a mulberry leaves quantitatively and qualitatively to enhance the cocoon production (Choudhury *et al*., 1991; Raman *et al*., 1995). It is well-known that, mainly quantity and quality of mulberry leaves influence the growth rate, developmental period, larval weight, low larval mortality, fecundity as well movement and competitive ability in the adults. Thus the nutritional level in mulberry leaves increase the larval growth and cocoon characters with enriched nutrients (Parra, 1991; Sengupta *et al*., 1992)

The supplementation of ascorbic acid (Vitamin-C) solution with different concentrations 0.5, 1.0 & 1.5% over control (untreated) batches showed significant increase in the larval growth and cocoon character compared to control (Miranda *et al*., 1998). Suprakash and Pal in 2002 also conducted and experiment with mulberry leaves dipped in Vitamin-B complex solution of different concentrations 0.5, 1 & 1.5 % and dried under shade and fed to *Bombyx mori* L. and 0.5% exhibited the increase in the larval weight and shell ratio. Similarly, propolis extracts (a honeybee product) yielded more cocoon weight with higher in silk production as well more numbers of eggs laid by the females over control batches (Nour *et al*., 1997). In the year 1994, Zannoon also reported the similar results using bee honey but cocoon silk content ratio and filament size was not affected.

Honey is the richest and natural nutrient comprising carbohydrates (82%), proteins, enzymes (e.g. Diastase, invertase, glucose oxidase, catalase, etc.), free amino acids, trace amounts of vitamin-B and vitamin- C and metals such as Cr, Co, Cu, Fe, Mn and Zn (Falco *et al*., 2003; Garcia *et al*., 2005; David Ball, 2007). It plays vital role in the growth and metabolism of *Bombyx mori* L. The lower concentration level respond greater over zero dose control is a powerful technique in the detection of concentration response in growth related studies (Stewart and Ruberg, 2000; Jan, 2005; Rani*et al*., 2011; Kavitha *et al*., 2011; Thulasi and Sivaprasad, 2014).

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The effect of honey on ethanol-induced increased vascular permeability changes was studied in the rat stomach. Ethanol produced concentration and time-dependent increase in the extravagation of Evans blue. Oral administration of honey (0.078–0.625 g/kg) 30 min before ethanol dose-dependently attenuated ethanol-induced increased vascular permeability. Similarly, sucralfate (0.031–0.250 g/kg) orally and allopurinol (0.025–0.050 g/kg) intravenously inhibited vascular permeability caused by ethanol and treatment with *N*-ethyl maleimide before sucralfate or allopurinol reduced their inhibitory effects. These results suggest that the protective effect of honey may be mediated through sulfhydryl-sensitive processes and it may also possess antioxidant properties. (Al-Swayeh *et al.,* 1997). It is also suggested that endogenous sulfhydryl may facilitate and mediate beneficial effects of gastro protective and antioxidant drugs.Keeping the above information in view, an investigation has been undertaken to know the influence of honey on biochemical composition and economic characters of Eri silkworm.

II. MATERIALS AND METHODS

➢ *Materials used for the Study*

In this experiment Castor variety "**Local pink**", Foliar formulations '**Honey'** and Eri silkworm strain "**Blue-Plain"** were selected.

➢ *Advantages of Honey*

Honey contains flavonoids; antioxidants which help reduce the risk of some cancers and heart disease. It reduces the ulcers and other gastrointestinal disorders.Due to its anti-bacterial, anti-fungal, anti-fungalproperties reduces the cough and irritation in throat. Also helps in the bone and immune health as well increase in the performance of athletics (Samarghandian *et al.,*2017; Yusof *et al.,* 2018: Raeessi *et al.,* 2011).

➢ *Composition of Honey*

Nutritional facts per 100g of honey

- Energy: 320 kcal
- Protein: 0 g
- Calcium: 13 mg
- Carbohydrate: 80 g
- Fat: 0 g
- Iron: 1.5 mg
- Natural sugars: 80 g
- Sodium: 17 mg
- Phosphorus: 5 mg
- Added sugar: 0g
- Potassium: 138 mg

➢ *Preparation of Honey Solution*

Honey at different concentrations was prepared using distilled water:

- **T1** = 1 % concentration
- **T2** = 2 % concentration
- **T3** = 3 % concentration
- **T4** = 4 % concentration
- **T5** = 5 % concentration
- **T6** = Distilled water control
- **T7** = Absolute control

➢ *Method of Application of Honey*

Castor leaves were dipped in different concentrations of honey and were shade dried. The treated leaves were fed to Eri silkworms once a day (first feed) during 4thand 5thinstar stages.

➢ *Disinfection of Rearing House and Equipment's*

The rearing appliances were cleaned, washed thoroughly and disinfected properly with five per cent bleaching powder solution one week before the commencement silkworm rearing (Dandin and Giridhar, 2010).

➢ *Procurement of Disease Free Layings*

Disease free layings of Eri silkworm was procured from College of Sericulture, University of Agricultural Sciences, Chintamani, were incubated at 25±1ºC and relative humidity of 75±5%.

➢ *Black Boxing and Brushing of Eri Silkworms*

Eri silkworm eggs (two disease free layings) were black boxed on the day of pin head stage to ensure uniform hatching by adopting black paper method. On the day of hatching, the eggs are exposed to diffused light and after two hours of hatching, the larvae were offered tender leaves of castor.

➢ *Eri Silkworm Rearing*

Eri silkworm rearing was conducted from day of brushing to spinning (Dayashankar, 1982) by using local pink variety of castor. The average temperature of 31.12ºC and relative humidity of 55.79% were recorded during rearing. The Eri silkworms after third moult *i.e.,* during fourth and fifth instar (50 larvae / replication) were used to study enrichment of castor leaves with honey on bio-assay of Eri silkworm.

➢ *Biochemical Analysis of Eri Silkworm*

Biochemical constituents in haemolymph, fat body and silk gland samples in 5th day of fifth instar larvae were analyzed. The collected samples were preserved in -20ºC and used for quantitative estimation using spectrophotometer and the obtained results were expressed in terms of mg/ml for haemolymph and mg/g for fat body and silk gland.

- *Protein Estimation Lowry's Method*

Total protein content in haemolymph**,** fat body and silk gland of silkworm were analyzed and calculated by adopting Lowry *et al.* (1951) procedure. Fat body and samples (0.1g) was homogenized in 10 ml of trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 20 minutes. Silk gland samples are crushed using distilled water and centrifuged at 3000 rpm for 10 minutes. One ml each of haemolymph, fat body and silk gland samples was added with 5 ml of protein reagent and 0.5 ml of Folin's reagent (1:1) and incubated for

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30 minutes at room temperature. Instead of supernatant, distilled water was used as a blank and BSA of 0.75 mg/ml was used as standard. The blue colour intensity was measured using spectrophotometer at 660 nm against blank. The results were expressed using standard graph and indicated in mg / ml for haemolymph and mg / g of wet tissue for fat body and silk gland.

- *Carbohydrate Estimation by Anthrone Method*

Total carbohydrate content in haemolymph**,** fat body and silk gland of silkworm was estimated by adopting the procedure of Anthrone method (Sadasivam and Manickam, 2008). Fat body and silk gland samples (0.1g) was homogenized in 10 ml of trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 20 min. Silk gland samples are crushed with using distilled water and centrifuged at 3000 rpm for 10 minutes. One ml each of haemolymph, fat body, silk gland samples was added with 4 ml of Anthrone reagent and boil for 8 minutes. In the place of supernatant, distilled water was used as a blank. The pale green colour intensity will be measured using spectrophotometer at 630 nm against blank. The results were showed using standard graph and expressed in mg / ml for haemolymph and mg / g of wet tissue for fat body and silk gland.

➢ *Economic Parameters*

- *Larval Weight*

Matured larval weight of fifth instar 5thday was recorded in each treatment, replication-wise.

- *Larval Duration (Days)*

Fifth instar and total larval duration were recorded in different treatments, replication-wise.

- *Cocoon Weight*

Cocoon weight was recorded by weighing cocoons individually using sensitive electronic balance.

- *Pupal Weight*

Pupal weight was recorded by weighing pupa individually using sensitive electronic balance.

- *Shell Weight*

Shell weight was recorded by removing floss layer and cutting open the cocoon to remove pupa and the last larval skin *i.e.,* exuvium.

- *Shell Ratio (%)*

The shell ratio was calculated using the following formula:

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

- *Silk Productivity (cg/day)*

The silk productivity was calculated using the formula:

$$\text{Silk productivity (cg/day)} = \frac{\text{Shell weight (cg)}}{\text{Fifth instar larval duration (days)}} \times 100$$

➢ *Statistical Analysis of Data*

The experimental data collected on Eri silkworm was analyzed statistically for test of significance using Fisher's method of Analysis of Variance (ANOVA) (Cochran and Cox, 2000) using SPSS statistical package.

III. RESULTS

➢ *Biochemical Composition in Three Tissues of Eri Silkworm*

- *Total Protein*

✓ *Haemolymph:*

Eri silkworms fed on honey supplemented castor leaf showed significant differences in protein content of haemolymph. Higher protein content was recorded at 5% concentration (88.20 mg/g) followed by 4% (66.78 mg/g) and 3% (56.74 mg/g). However, protein content was lower in 1% (49.37 mg/ml) (Table 1 & Figure 1, 3**)**.

✓ *Fat Body:*

Total protein content in the fat body of Eri silkworm showed highly significant differences among different treatments. Highest protein content was recorded in the batch of Eri silkworms fed with 5% concentration of honey (13.43 mg/g) followed by 4% (12.49 mg/g) and 3% (11.80 mg/g), while lowest protein content was found with absolute control (9.182 mg/g) (Table 1 & Figure 1, 3**)**.

✓ *Silk Gland:*

Highly significant differences were noticed in protein content of the Eri silkworm's silk gland. Total Protein content was more in the batch of worms fed with 5% concentration honey supplemented castor leaves (33.68 mg/g), followed by 4% (28.87 mg/g), 3% (26.62 mg/g) and distilled water control (24.25 mg/g), while less content was recorded when worms did not receive either honey supplemented or distilled sprayed leaves (20.30 mg/g) (Table 1 & Figure 1, 3**)**.

- *Total Carbohydrate*

✓ *Haemolymph:*

Carbohydrate of the Eri silkworm's haemolymph showed highly significant differences among the treatments. The highest carbohydrate content was recorded in 5% concentration (16.24 mg/ml) followed by 4% (10.90 mg/ml), 3% (9.744 mg/ml) and 2% (8.557 mg/ml). However, it was lowest with 1% concentration (8.054 mg/ml) (Table 1 & Figure 2, 4).

✓ *Fat Body:*

Carbohydrate content in fat body of Eri silkworm also showed highly significant differences among the treatments. Highest amount of carbohydrate content was noticed when the worms were fed on 5% concentration honey

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supplemented leaves (17.62 mg/g) followed by 4% (14.05 mg/g), 3% (12.63 mg/g) and 2% (12.57 mg/g). The lowest amount of carbohydrate was found in absolute control (10.49 mg/g) (Table 1 &Figure 2, 4).

✓ *Silk Gland:*

The carbohydrate content in silk gland of Eri silkworm showed non-significant differences among the treatments. It ranged from 6.320 (absolute control) to 7.989 mg/g (5% concentration) (Table 1 &Figure 2, 4).

➢ *Rearing Parameters of Eri Silkworm*

✓ *Matured Larval Weight and Larval Duration:*

Significant differences were noticed in matured larval weight, when the worms were reared on castor leaves supplemented with different concentrations of honey. Matured larval weight was highest in the batch of worms fed on castor leaves supplemented with honey at 5% concentration (8.566 g) followed by 4% (7.643 g), 3% (7.438 g), 1% (6.938 g) and distilled water control (6.715 g). However, matured larval weight was lowest in 2% (6.263 g). The Eri worms fed on castor leaves supplemented with honey at different concentration recorded 6 and 19 days of fifth instar and total larval duration, respectively (Table 2 and Figure 5, 7).

➢ *Cocoon Parameters of Eri Silkworm*

- *Cocoon Weight*

Cocoon weight differs significantly among different concentrations of honey. Highest cocoon weight was registered when the worms fed on castor leaves supplemented with honey at 5% concentration (2.777 g) followed by 3% (2.561 g), 4% (2.545 g), 1% (2.436 g) and absolute control (2.422 g). However, least cocoon weight was observed in 2% of honey (2.304 g) (Table 2 & Figure 6, 8).

- *Pupal Weight*

Eri silkworms fed on castor leaves supplemented with honey at different concentrations differed significantly with more being in 5% (2.489 g) followed by 4% (2.361 g), 1% (2.316 g) and 3% (2.197 g). However, less pupal weight was noticed in 2% (2.057 g) (Table 2 & Figure9).

Table 1 Total Protein and Total Carbohydrate Contents in Different Tissues of Eri Silkworm Reared on Honey Supplemented Castor Leaves.

Treatment	Total Protein			Total Carbohydrate		
	Haemolymph (mg/ml)	Fat body (mg/g)	Silk gland (mg/g)	Haemolymph (mg/ml)	Fat body (mg/g)	Silk gland (mg/g)
T-1: 1% Conc.	49.37 ± 1.763 (-1.438)	10.49 ± 0.396 (12.46)	22.93 ± 1.237 (11.46)	8.054 ± 0.354 (-0.975)	11.36 ± 0.552 (7.65)	6.515 ± 0.196 (2.993)
T-2: 2% Conc.	51.29 ± 1.610 (2.359)	11.07 ± 0.127 (17.055)	24.10 ± 2.061 (15.76)	8.557 ± 0.144 (4.955)	12.57 ± 0.503 (16.54)	6.802 ± 1.160 (7.086)
T-3: 3% Conc.	56.74 ± 3.767 (11.73)	11.80 ± 0.396 (22.186)	26.62± 0.119 (23.74)	9.744 ± 0.500 (16.53)	12.63 ± 0.602 (16.94)	7.076 ± 0.564 (10.68)
T-4: 4% Conc.	66.78 ± 5.534 (29.41)	12.49 ± 0.251 (26.48)	28.87 ± 0.073 (29.68)	10.90 ± 0.588 (22.39)	14.05 ± 0.557 (25.33)	7.389 ± 0.097 (14.56)
T-5: 5% Conc.	88.20 ± 9.088 (43.21)	13.43 ± 0.466 (31.63)	33.68 ± 0.915 (39.72)	16.24 ± 0.535 (49.91)	17.62 ± 1.781 (40.46)	7.989 ± 0.562 (20.891)
T-6: Distilled water control	51.14 ± 6.552 (2.072)	10.66 ± 0.219 (13.86)	24.25 ± 0.401 (16.28)	8.459 ± 0.302 (3.85)	12.39 ± 0.395 (15.33)	6.613 ± 0.733 (4.43)
T-7: Absolute control	50.08 ± 0.095	9.182 ± 0.241	20.30 ± 0.573	8.133 ± 0.397	10.49 ± 0.890	6.320 ± 0.245
F-test	5.086*	19.207**	19.001**	47.071**	7.006**	0.893^{NS}

±: Standard error (SE) values*: Significant at $p \leq 0.05$**: Significant at $p \leq 0.01$ NS: Non-significant ( ): % change values over absolute control

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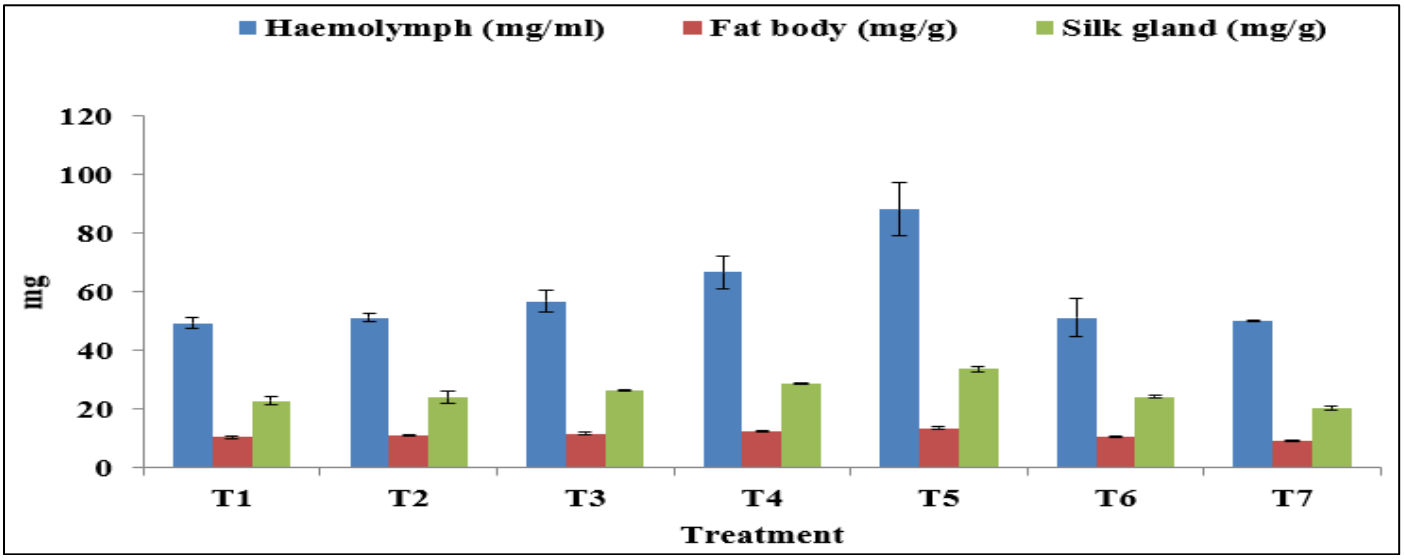

Fig 1 Total Protein Content in Different Tissues of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

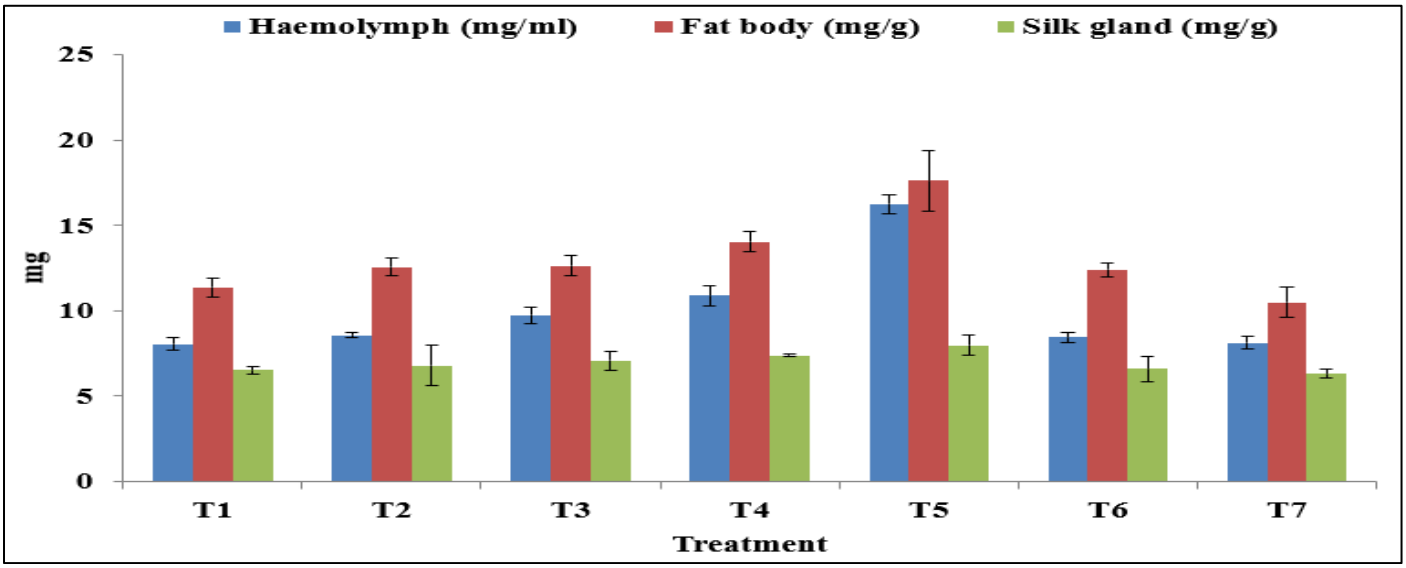

Fig 2 Total Carbohydrate Content in Different Tissues of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

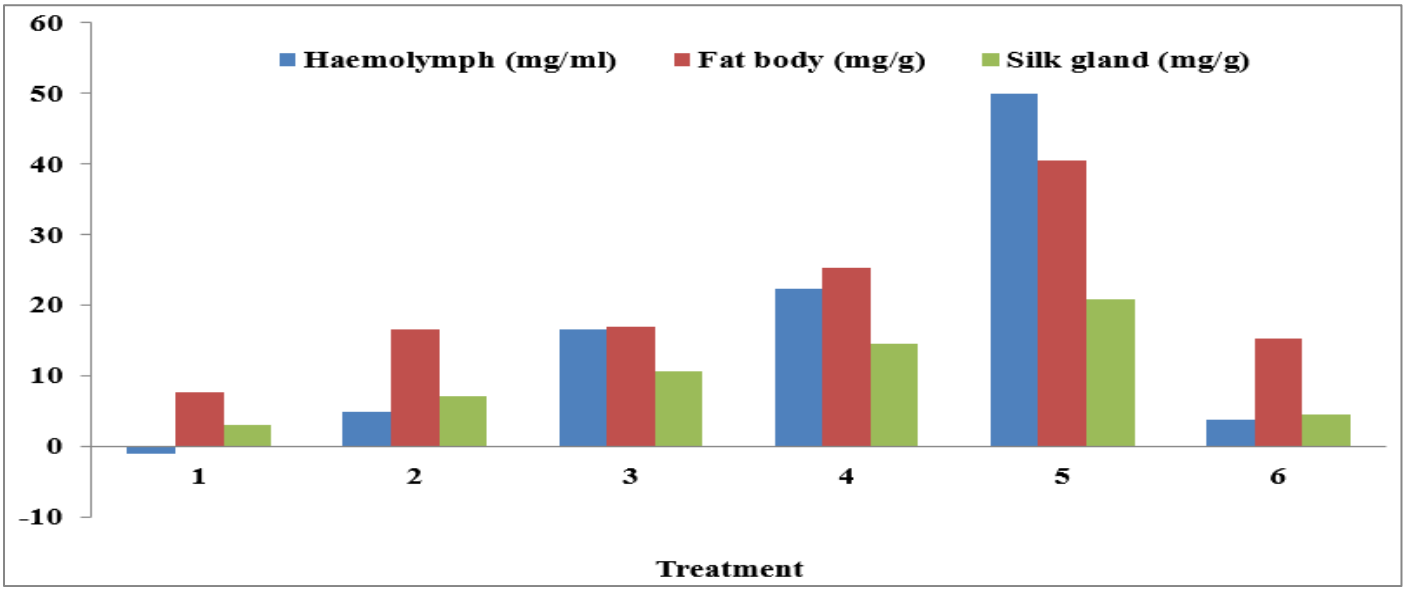

Fig 3 Percent Change of Total Protein in Different Tissues of Eri Silkworm Reared on Honey Supplemented Castor Leaf over Absolute Control.

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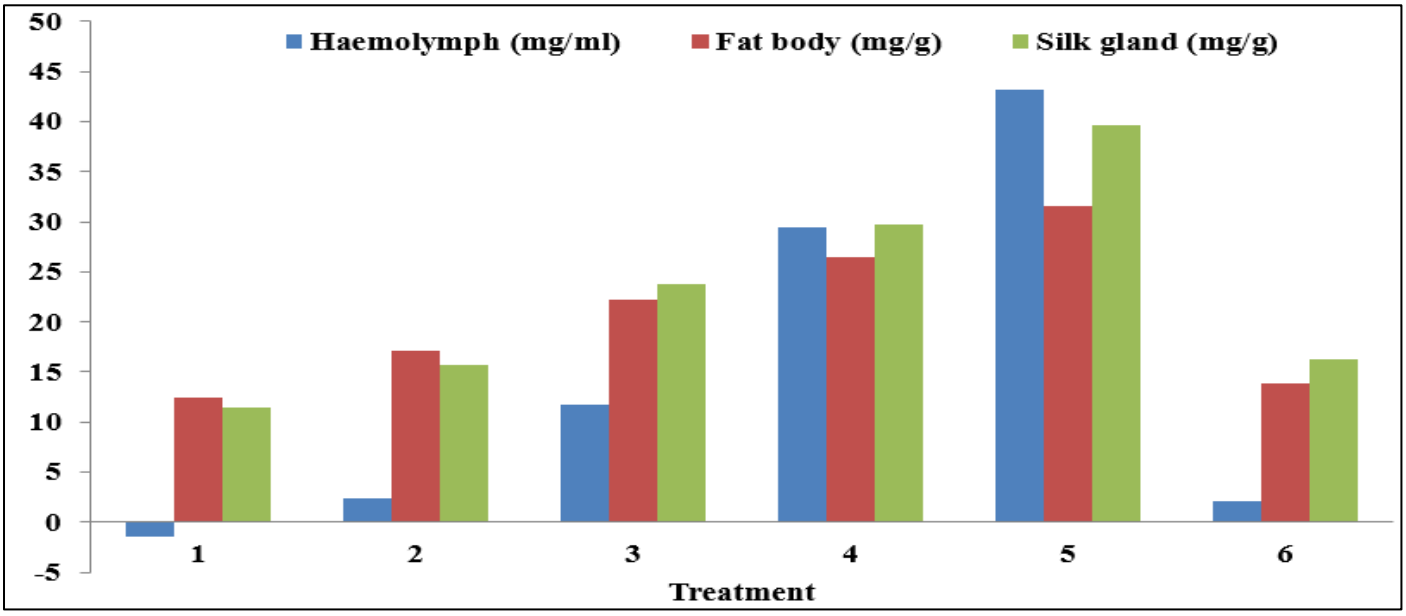

Fig 4 Percent Change of Total Carbohydrate in Different Tissues of Eri Reared on Honey Supplemented Castor Leaf over Absolute Control.

- *Shell Weight*

Shell weight was found significant when Eri worms fed on castor leaves supplemented with honey at different concentrations. Higher shell weight was registered in 3% (0.364 g) followed by 5% (0.288 g), 1% (0.279 g) and absolute control (0.266 g), whereas lower value was found with at 4% (0.184 g) (Table 2 & Figure 10).

- *Shell Ratio*

Significant differences were observed among the treatments with regard to shell ratio with higher being in 3% concentration of honey (14.21 %) followed by 1% (11.45 %), absolute control (10.98 %), distilled water control (10.73 %) and 2% (10.71 %). However, shell ratio was lower when the larvae were fed on castor leaves supplemented with 4% of honey concentration (7.421 %) (Table2 & Figure 11).

- *Silk Productivity*

Silk productivity showed significant differences when the worms were reared on different concentrations of honey supplemented castor leaves. Silk productivity was highest in the worms where they were fed on castor leaves supplemented with 3% honey concentration (6.063 cg) followed by 5% (4.792 cg), 1% (4.642 cg) and absolute control (4.434 cg). However, 4% concentration of honey (3.071 cg) registered lower silk productivity when compared to the other honey treated batches (Table 2 & Figure 12).

In the overall economic parameters, among the different concentrations, silkworm batch with 5% concentration showed better performance with respect to larvae, cocoon and pupal weight when compared to other concentration batches over controls. But, in case of silk quantity, batches fed with 3% concentration exhibited notable increase followed by other treated batches over controls. This evidently states that, as the nutrients increases in pupae silk material decreases in 5% treated batches. Whereas, in 3% treated batches nutrients decreased in pupae silk material increased (Figure 13).

Table 2 Economic Parameters of Eri Silkworm Reared on Honey Supplemented Castor Leaf

Treatment	Matured larval weight (g)	Cocoon Weight (g)	Pupal Weight (g)	Shell Weight (g)	Shell Ratio (%)	Silk productivity (cg/day)
T-1: 1% Conc.	6.938 ± 0.017 (4.886)	2.436 ± 0.038 (0.574)	2.316 ± 0.060 (6.908)	0.279 ± 0.022 (4.659)	11.45± 1.001 (4.104)	4.642 ± 0.373 (4.480)
T-2: 2% Conc.	6.263 ± 0.009 (-5.34)	2.304 ± 0.009 (-5.121)	2.057 ± 0.018 (-4.812)	0.247 ± 0.022 (-7.692)	10.71± 0.924 (-2.52)	4.117 ± 0.367 (-7.699)
T-3: 3% Conc.	7.438 ± 0.014 (11.281)	2.561 ± 0.005 (5.427)	2.197 ± 0.034 (1.866)	0.364 ± 0.029 (26.923)	14.21± 1.160 (22.27)	6.063 ± 0.485 (26.867)

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T-4: 4% Conc.	7.643 ± 0.048 (13.661)	2.545 ± 0.012 (4.833)	2.361 ± 0.017 (8.682)	0.184 ± 0.011 (-44.56)	7.421± 0.425 (-47.95)	3.071 ± 0.178 (-44.382)
T-5: 5% Conc.	8.566 ± 0.049 (22.965)	2.777 ± 0.011 (12.783)	2.489 ± 0.021 (13.378)	0.288 ± 0.016 (7.638)	10.36± 0.589 (-5.98)	4.792 ± 0.267 (7.470)
T-6: Distilled water control	6.715 ± 0.019 (1.727)	2.347 ± 0.012 (-3.195)	2.095 ± 0.026 (-2.911)	0.252 ± 0.016 (-5.55)	10.73± 0.718 (-2.32)	4.192 ± 0.261 (-5.772)
T-7: Absolute control	6.599 ± 0.097	2.422 ± 0.011	2.156 ± 0.029	0.266 ± 0.030	10.98± 1.220	4.434 ± 0.499
F-test	283.81**	85.43**	23.33**	6.068**	5.085**	6.069**

±: Standard error (SE) values*: Significant at $p \leq 0.05$ **: Significant at $p \leq 0.01$( ): % change values over absolute control 5th instar larval duration: 6 days Total larval duration: 19 days

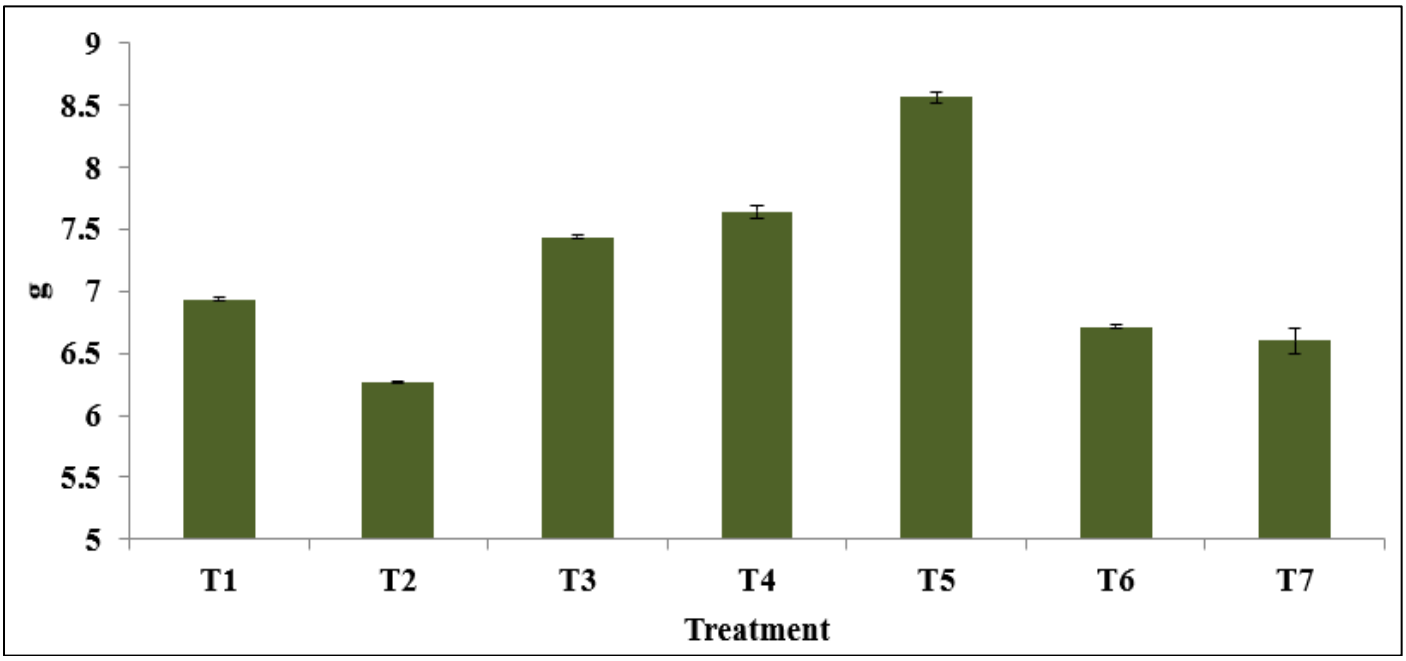

Fig 5 Matured Larval Weight of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

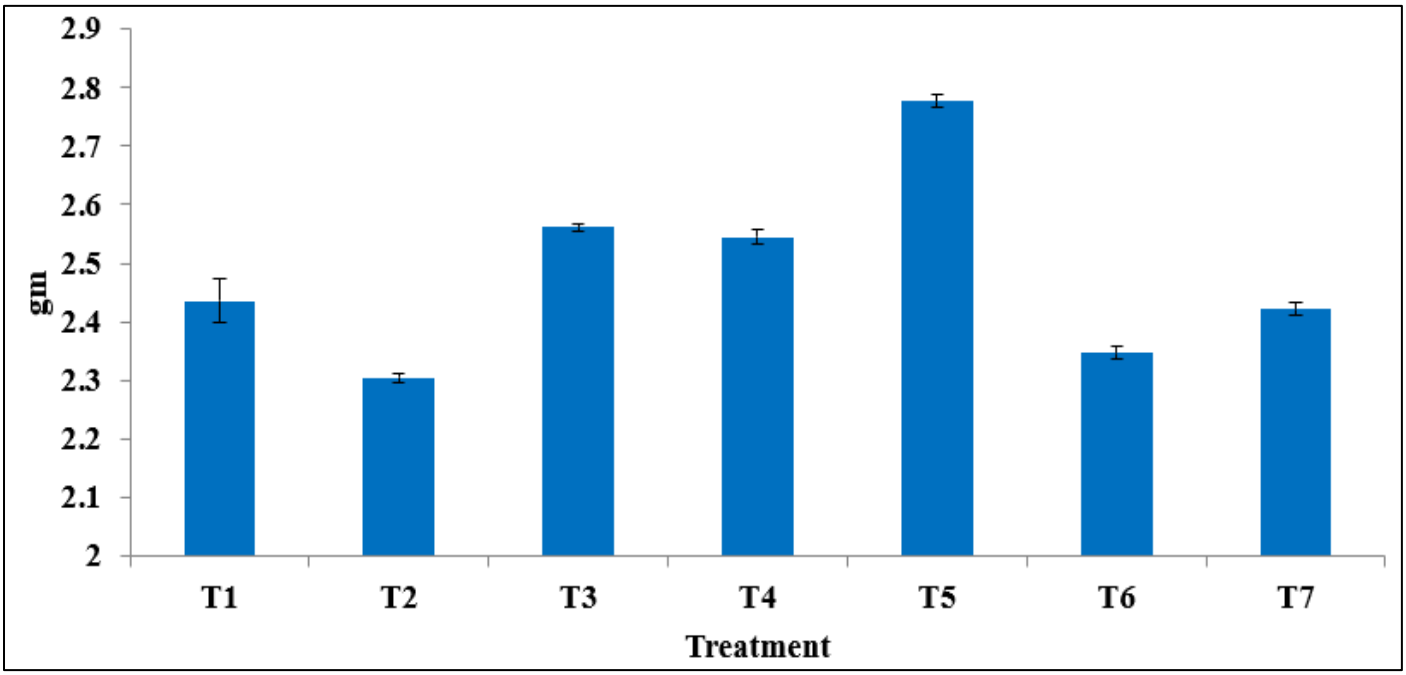

Fig 6 Cocoon Weight of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

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Fig 7 Eri Silkworms Reared on Honey Supplemented Castor Leaf

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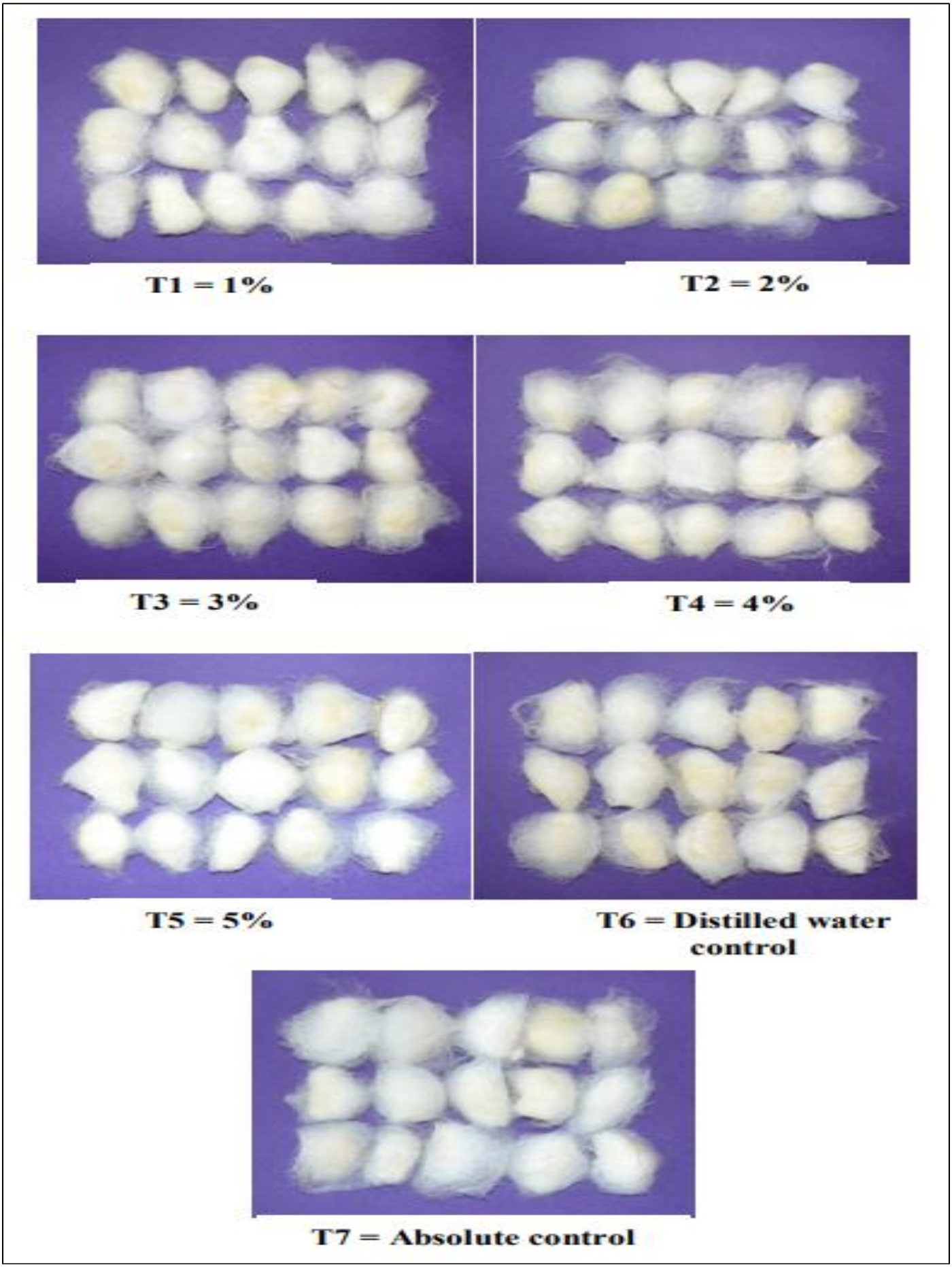

Fig 8 Cocoons of Eri Silkworms Reared on Honey Supplemented Castor Leaf

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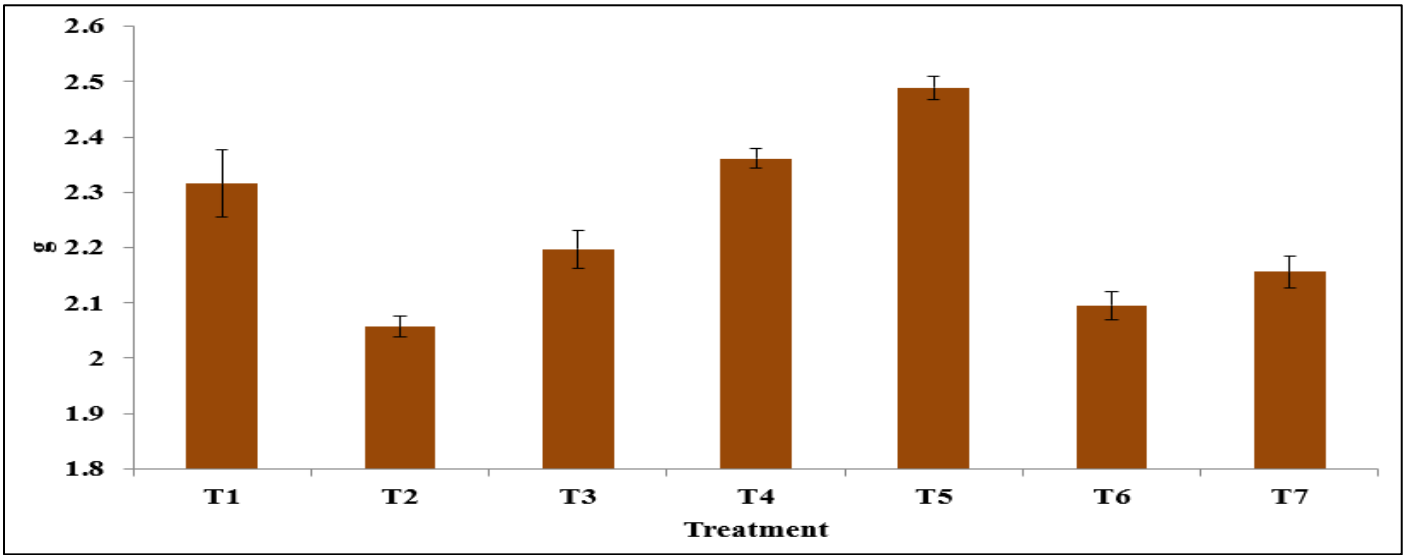

Fig 9 Pupal Weight of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

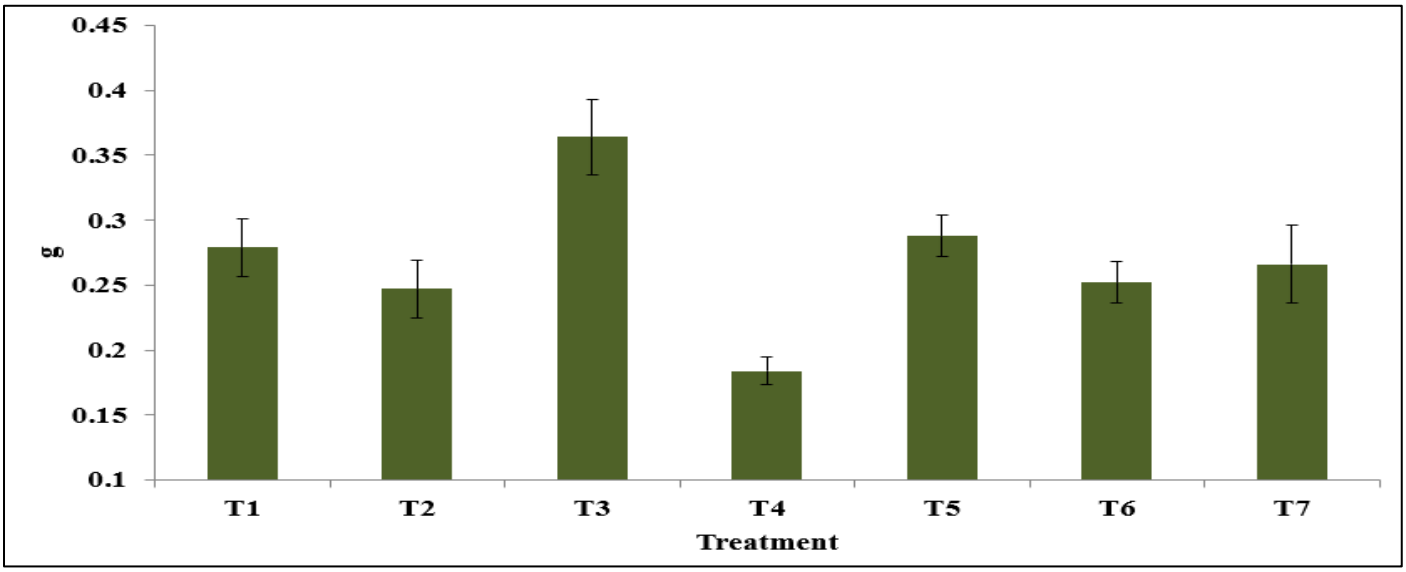

Fig 10 Shell Weight of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

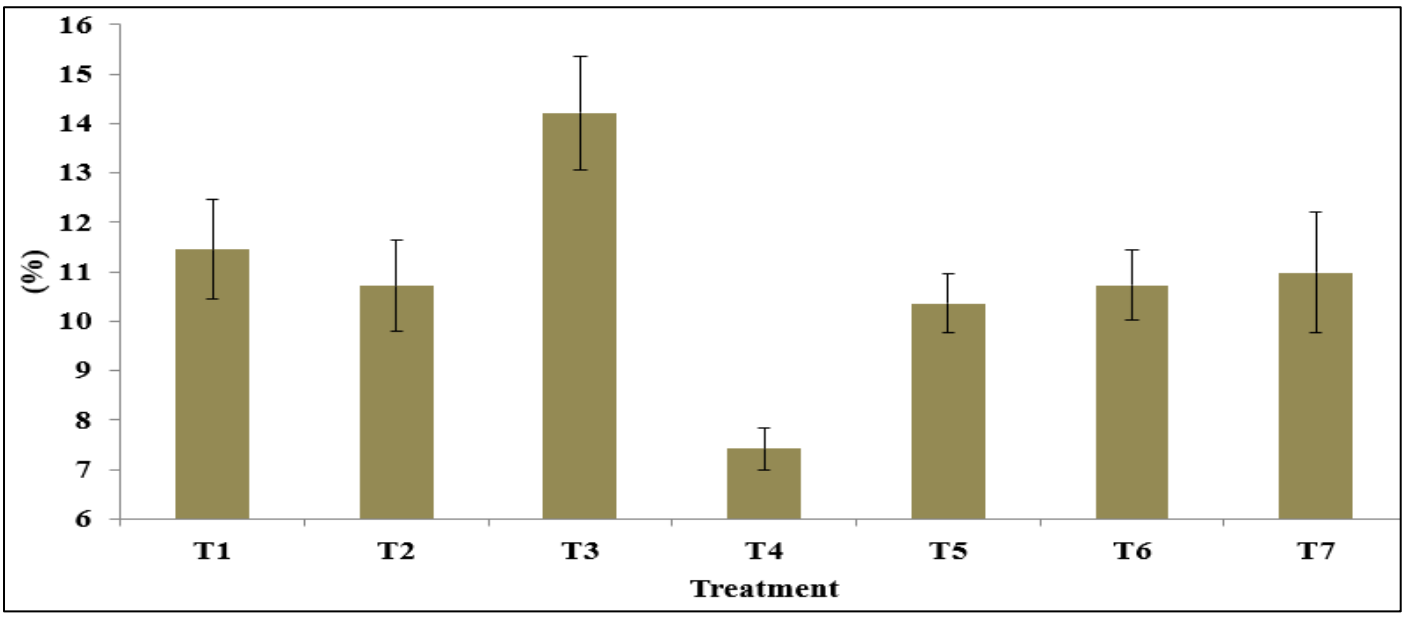

Fig 11 Shell Ratio of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

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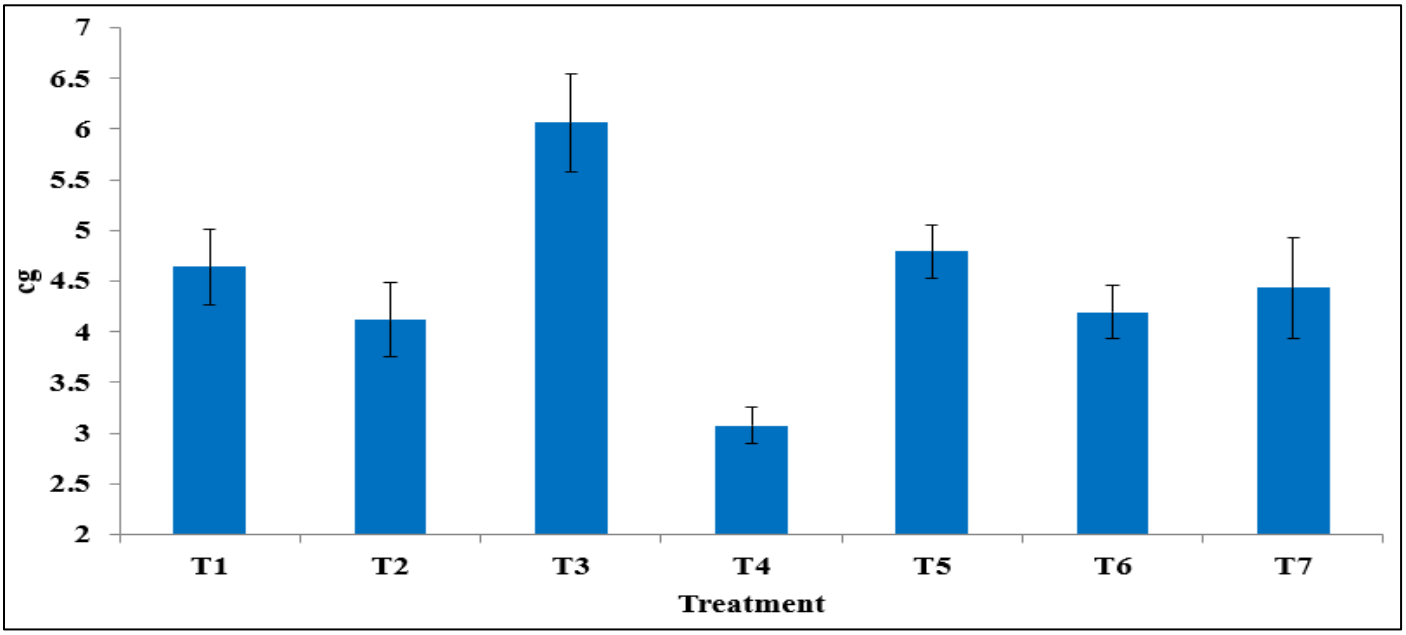

Fig 12 Silk Productivity of Eri Silkworm Reared on Honey Supplemented Castor Leaf.

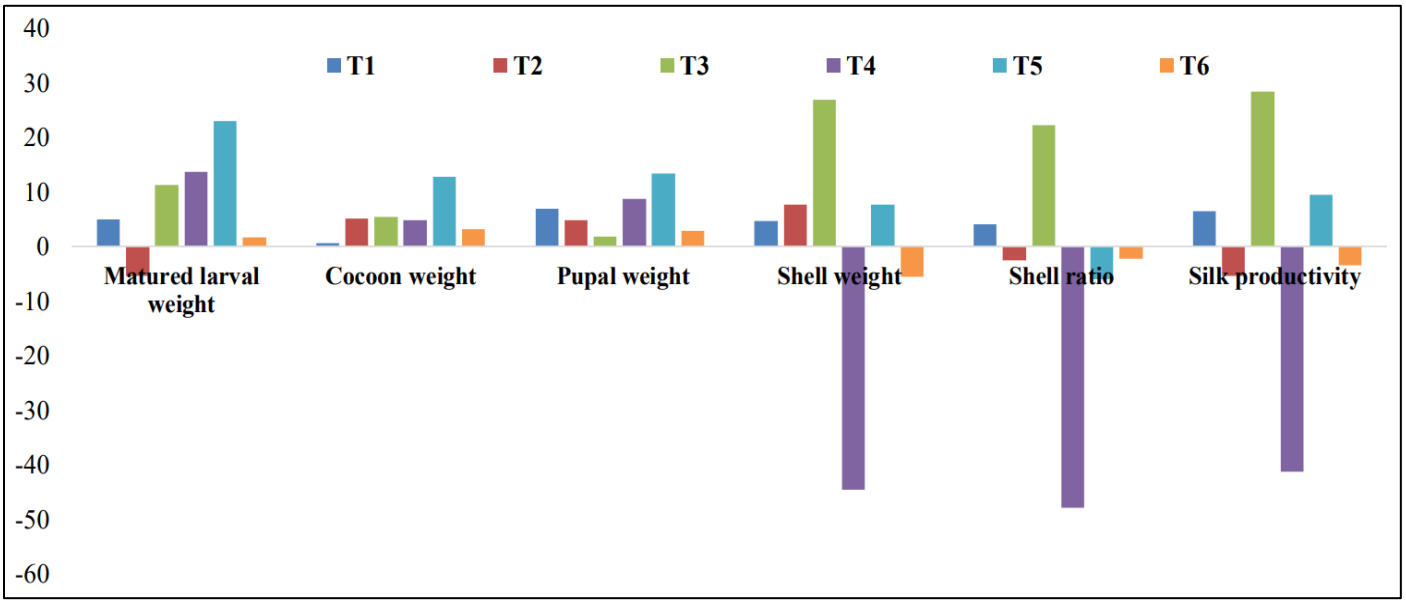

Fig 13 Percent Change of Economic Parameters in Different Tissues of Eri Silkworm Reared on Honey Supplemented Castor Leaf Over Absolute Control.

IV. DISCUSSION

Total protein and total carbohydrate contents in different tissues of Eri silkworm namely haemolymph, fat body and silk gland were more in worms reared on 5% concentration of honey supplemented castor leaves and less in both the controls. Thulasi and Sivaprasad (2014) reported that, honey caused significant improvement in the whole silk gland protein (SGP), it is necessary to find as to which region of silk gland (SG) responds more effectively to this exogenous factor. Hence, protein levels were estimated separately in the three regions namely, anterior silk gland (ASG), middle silk gland (MSG) and posterior silk gland (PSG). In the experiment results were observed that, the highest protein levels in MSG followed by ASG and PSG respectively.

Similarly, the rearing parameters and economic traits of Eri silkworm, in Matured larval weight was higher in the batches of Eri silkworms reared on honey supplemented castor leaf with 5% concentration. The matured larval weight increases with increase in concentration of honey. The economic parameters like cocoon weight, pupal weight, shell weight, shell ratio and silk productivity of the eri silkworm were higher when worms reared on honey supplemented castor leaf with 5% and 3% concentrations. As reported earlier, the impact of honey on larval growth is attributable to its presence of vitamins and minerals (Bhattacharya and Kaliwal, 2005a, 2005b; Rajabi *et al.*, 2006; Kavitha *et al.*, 2012).

The determination of gland-body ratio (GBR) by comparative analysis, resulted increase in the larval weight

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and silk gland during 5th instar larvae, this indicates the positive development in view of silk productivity (Sailaja and Sivaprasad, 2010). Achieving higher GBR can be done by enriching mulberry leaves with the potential exogenous modulators and nutrients; honey is identified as one such modulator. Singh in 1960 reported that, with prepared concentrations of royal jelly increased the larval and cocoon weight. El-Karaksy (1979) also concluded that, the use of royal jelly with yeast as food additive gave the heaviest larval, cocoon weight, silk gland and increase in the number of deposited egg by females of *Bombyx mori* L. and *Philosamia ricini*. El-Karaksy *et al.*, (1990) also found that, use of royal jelly at different concentrations of 2 and 4% enhanced the silk production and fecundity.

El-Sayed (1999) reported that the mixture of honey and black cumin seeds increased silk production and the number of deposited eggs by female and exhibited highest larval weight, pupal weight, dry silk gland and increase in the total protein content in silk gland. El-Massarawy (1995) and Gad (2006) found that silkworms fed with mulberry leaves supplemented with propolis extract yielded more cocoon weight, increased percentage of silk content and more fecundity than those obtained from control.

V. CONCLUSION

In the current investigation, it is evidently shows that, the total protein and carbohydrate contents in haemolymph, fat body and silk gland of Eri silkworm were significantly higher in the batch of silkworms reared on castor leaves supplemented with honey at 5% concentration. As well economic parameters of Eri silk worm namely matured larval weight; cocoon weight, pupal weight and shell weight were significantly more with 5% concentration of honey, while shell ratio and silk productivity was higher with 3% concentration of honey. Thus, the results of the study inferred that, the supplementation of 3% to 5% concentration of honey to castor leaves showed improvement in biochemical constituents, rearing and cocoon parameters of Eri silkworm. This experimental report provides the valuable guidelines and economical strategies to the Eri culture farmers.

- *Conflict of Interest*

There is no such conflict of interest was reported by authors with respect to this manuscript.

- *Author Contribution*

All authors provided critical feedback and helped to shape the research, analysis and approval of final manuscript.

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